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Tyrosine Hydroxylase Activity and Catecholamine Biosynthesis in the Adrenal Medulla of Rats during Stress[1]

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ABSTRACT
Chronic hypotension and hypoglycemia are known to increase the capacity for catecholamine biosynthesis in the rat adrenal medulla by increasing the maximal velocity of the rate-limiting enzyme, tyrosine hydroxylase (TH). The present report indicates that the gradual increase in maximal TH activity is preceded by a more rapid increase in the affinity of TH for its pterin cofactor. These short-term alterations in adrenal TH activity are related to the severity of the stress, associated with parallel changes in catecholamine biosynthesis, and prevented by prior adrenal denervation. In contrast, cold exposure, which leads to comparable long-term increases in adrenal TH activity, does so without causing a prior activation of TH. However, adrenal TH is activated by acute cold exposure if the sympathetic nerves, that normally are stimulated during cold, are destroyed previously by 6-hydroxydopamine treatment. These and other observations suggest that alterations in adrenal TH activity vary according to the type and duration of physiological stress, and may be mediated by temporally distinct processes.

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Stimuli that increase the release of CAs from the sympathoadrenal system also increase the rate-limiting step in CA biosynthesis, the hydroxylation of tyrosine. Among the mechanisms by which CA synthesis and secretion are coupled, two types of changes in TH seem to be particularly important. Prolonged increases in CA release lead to a gradual elevation in the maximal velocity of the TH-catalyzed reaction, owing to the apparent formation of additional enzyme molecules. This effect has been observed in response to such chronic physiological stressors as insulin-induced hypoglycemia (Patrick and Kirshner, 1971; Weiner and Mosimann, 1970), phenoxybenzamine-induced hypotension (Dairman and Udenfriend, 1970; Thoenen *et al.*, 1969a) and exposure to cold (Chuang and Costa, 1974; Hoeldtke *et al.*, 1974; Kvetnansky *et al.*, 1971). In addition, short-term increases in CA release lead to a rapid activation of TH, which often expresses itself as an increased affinity of TH for its pterin cofactor. This effect has been demonstrated within the adrenal medulla by treatments such as decapitation (Masserano and Weiner, 1979), electroconvulsive shock (Masserano *et al.*, 1981) and formalin-induced tissue damage (Masserano and Weiner, 1981).

These two mechanisms for increasing CA synthesis are not mutually exclusive, nor do they always occur in association with one another. For example, in a brief report we noted that the physiological stress associated with insulin-induced hypoglycemia provoked both an acute activation of TH and apparent enzyme induction within the adrenal medulla of rats, whereas cold exposure appeared to elicit only the longer-term response (Fluharty *et al.*, 1983). The present investigations extend that recent work by providing a more complete description of the short- and long-term changes in adrenal TH activity and CA biosynthesis that occur in response to glucoprivation, hypotension and cold.

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Methods

Animals. Male Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) weighing 250 to 400 g were used. Rats were housed either individually or in pairs in wire-mesh cages in a temperature-controlled room (22–23°C). Lights were on in that room between 8:00 A.M. and 8:00 P.M. Purina Chow pellets and tap water were available continuously unless otherwise noted. In some animals both adrenal glands were denervated by the supplier before shipping. We verified the completeness of adrenal denervation by determining that the animals did not display an appropriate hyperglycemia after i.p. injection of 500 mg/kg of 2-deoxy-D-glucose; this response is known to be mediated exclusively by the sympathoadrenal system (Brown and Bachrach, 1959).

Procedures.

All experiments were begun between 10:00 A.M. and 1:00 P.M.

Hypotension. The effect of drug-induced arterial hypotension on adrenal TH activity was studied in three experiments. First, we administered phenoxybenzamine (20 mg/kg i.p.), an *alpha* adrenergic receptor

ABBREVIATIONS: CA, catecholamine; TH, tyrosine hydroxylase; 6-MPH$_4$, 6-methyl-5,6,7,8-tetrahydropterin HCl.

blocking agent (Nickerson and Hollenberg, 1967), and sacrificed the rats 30 min later. Alternatively, isoproterenol (0.33 mg/kg s.c.), a *beta* adrenergic receptor agonist (Leenen and McDonald, 1974), was given and rats were sacrificed 15 min later. Finally, more prolonged hypotension was produced by giving animals phenoxybenzamine on 2 successive days and sacrificing them 24 hr after the second injection.

Glucoprivation. The effect of drug-induced glucoprivation on adrenal TH activity or CA biosynthesis was studied in three experiments. In the first, acute hypoglycemia was produced by administering insulin in pharmacological doses (Iletin, 5–20 U/kg s.c.). In the second, we administered 2-deoxy-D-glucose (500 mg/kg i.p.), a drug that inhibits glycolysis in all cells including brain (Woodward and Hudson, 1954). Some animals in each experiment were food-deprived for 16 hr before the treatments, to increase their severity. Rats were sacrificed 1 hr after the injections and blood samples were taken just before the adrenal glands were removed, for later analysis of plasma glucose concentration (Beckman glucose analyzer; Beckman Instruments, Fullerton, CA).

Finally, chronic hypoglycemia was produced either by administering regular insulin (20 U/kg s.c.) or long-lasting protamine-zinc insulin (4–8 U s.c.) each day for 4 days. In either case, rats were sacrificed 24 hr after the last injection. To increase the intensity of the treatments, some animals were deprived of food 2 hr before and 1 hr after each injection.

Cold. Rats were placed in a cold room (ambient temperature, 5°C) for various times ranging from 1 hr to 3 weeks. To increase the severity of this stress, some animals were shaved beforehand whereas other rats received the CA neurotoxin 6-hydroxydopamine (100 mg/kg s.c.) 4 days before cold exposure; this treatment produces substantial degeneration of sympathetic nerve terminals without damaging chromaffin cells of the adrenal medulla (deChamplain, 1971).

Tissue Analyses

Assay of TH activity. Animals were anesthetized deeply with Equithesin at the conclusion of each experiment and their adrenal glands were removed rapidly, dissected at 4°C and then stored at −70°C for 1 to 5 days before analysis. Soluble TH activity was assayed by a modification of the coupled decarboxylase assay of Waymire and colleagues (1971). Adrenal glands were homogenized in 1 ml of ice-cold 50 mM Tris-acetate buffer, pH 6.0, and centrifuged at 39,000 × *g* for 30 min. The resulting supernatant then was passed over a Sephadex G-25 column (12 cm × 0.9 cm) to remove endogenous CAs that otherwise might inhibit TH activity. The column was washed with 10 ml of homogenization buffer and then TH protein was eluted with 3.0 ml of the same buffer. This eluate was found to contain 100% of the initial TH activity (as determined in the presence of excess cofactor) and less than 2% of the total adrenal CA content. Moreover, the apparent K_m for pterin cofactor was lowered significantly but the apparent V_{max} was not (see table 1), an observation consistent with the presumed competition between cofactor and CAs for regulation of TH activity (Spector *et al.*, 1967).

Samples or Tris-acetate buffer (*i.e.*, blanks containing no tissue) then were incubated in triplicate at 37°C for 10 min in 0.2 M Tris-acetate buffer (usually pH 6.7; see below) in the presence of 75 μM L-[1-^{14}C]tyrosine (specific activity, 50–60 Ci/mol), 6-MPH$_4$, catalase, dihydroxypteridine reductase (partially purified from rat liver) and nicotinamide adenine dinucleotide, reduced form. The resulting L-[1-^{14}C]dopa subsequently was decarboxylated by addition of an excess of L-aromatic amino acid decarboxylase (partially purified from hog kidney) in the presence of 0.1 M Tris-acetate buffer (to bring the final pH of the reaction mixture to 6.8), pyridoxal-5′-phosphate and 3-iodo-L-tyrosine (to inhibit further hydroxylation). Finally, the liberated $^{14}CO_2$ was trapped in tissue solubilizer and quantified using liquid scintillation spectrometry. All experiments included within assay controls.

Using these procedures, we found that formation of $^{14}CO_2$ was linear both with time of the hydroxylation reaction and with amount of protein. Moreover, the direct decarboxylation of [^{14}C]tyrosine appeared to be minimal because formation of $^{14}CO_2$ was eliminated either by deleting 6-MPH$_4$ from the mix or by adding 3-iodo-L-tyrosine.

To improve our ability to detect increases in TH activity, we made use of preliminary studies in which pH and 6-MPH$_4$ concentrations were varied. We found that TH activity of adrenals from anesthetized control animals exhibited a rather broad pH curve, with very little change in activity from pH 5.7 to 6.2 and a small decline in activity thereafter (table 2). In contrast, when animals were sacrificed by decapitation, a procedure known to activate TH (Masserano and Weiner, 1979), the enzyme exhibited a pH optimum at 6.7 (table 2). Consequently, in the present experiments we removed adrenals from anesthetized animals and incubated them at pH 6.7 so that short-term increases in TH activity might be apparent more readily. Furthermore, because activation of TH in our assay system generally is expressed as an increase in affinity for cofactor, we measured TH in the presence of subsaturating concentrations of cofactor (0.1 mM 6-MPH$_4$) to detect activation of the enzyme (Fluharty *et al.*, 1983; Masserano and Weiner, 1979) and used saturating concentrations (10 mM 6-MPH$_4$) to measure changes in maximal enzyme activity.

***In vivo* CA biosynthesis.** The biosynthesis of CAs was assessed *in vivo* by a modification of methods described previously (Carlsson *et al.*, 1972). Animals were treated with the aromatic amino acid decarboxylase inhibitor RO 4-4602 (100 mg/kg i.p.) and were sacrificed 1 hr later. The left adrenal gland was removed rapidly and dissected free of fat. Dopa was measured using high-performance liquid chromatography, as described below. Under these conditions dopa accumulation was linear for 90 min.

Catechol content. Dopa and CA content were measured by a modification of previous methods (Keller *et al.*, 1976). Tissue was weighed and homogenized in 0.1 N perchloric acid containing 0.2 mM sodium metabisulfite. After a 10-min centrifugation at 15,000 × *g*, an appropriate volume of supernatant (100–300 μl) was combined with enough 0.1 N perchloric acid to make 300 μl total volume. An internal standard, 3,4-dihydroxybenzylamine, then was added, the pH was brought to 8.6 with Tris buffer and the CAs were absorbed onto alumina. After washing several times with buffered water, the CAs were eluted with 0.1 N perchloric acid and injected onto the liquid chromatographic column. Separation of dopa and CAs was achieved using a C18 reverse phase column with the mobile phase consisting of an aqueous solution of 0.1 M citric acid, 0.05 mM EDTA, 0.0975 mM

TABLE 1

Effect of sephadex G-25 chromatography on adrenal TH activity

Results are mean values from four separate experiments ± S.E.M. Adrenal TH activity was assayed in the presence of subsaturating (0.3 mM) and saturating (3.0 mM) concentrations of the cofactor (6-MPH$_4$) and is expressed in picomoles per milligram of protein per minute.

	Subsaturating (6-MPH$_4$)	Saturating (6-MPH$_4$)
Control	150.0 ± 11.0	820.0 ± 26.0
Sephadex-treated	265.0 ± 14.0*	915.0 ± 79.0
% Control	177	112

* P < .05, in comparison with control values.

TABLE 2

Effect of decapitation on the pH characteristics of adrenal TH activity

Results are mean values from four separate experiments ± S.E.M. Adrenal TH activity was assayed in the presence of subsaturating (0.3 mM) concentrations of the cofactor (6-MPH$_4$) and is expressed as picomoles per milligram of protein per minute.

pH	Control	Decapitation
5.7	285 ± 17	320 ± 15
5.9	270 ± 30	330 ± 15
6.2	285 ± 20	460 ± 10 (61%)*
6.5	225 ± 20	385 ± 15 (71%)*
6.7	215 ± 20	475 ± 15 (121%)*

* Numbers in parentheses represent statistically significant increases in TH activity, when compared to control values (P < .05).

octyl sulfate and 7.5% acetonitrile. Detection was based on the current produced when the separated compounds were oxidized during exposure to a glassy carbon electrode (+0.72 V *vs.* Ag/AgCl reference electrode). The order of elution was norepinephrine, dopa, epinephrine and the standard, and recovery of dopa and CAs from the alumina varied between 60 and 75%.

Results

Long-term effects. In confirmation of previous reports (Chuang and Costa, 1974; Dairman and Udenfriend, 1970; Hoeldtke *et al.*, 1974; Kvetnansky *et al.*, 1971; Patrick and Kirshner, 1971; Thoenen *et al.*, 1969a; Weiner and Mosimann, 1970), chronic hypotension, glucoprivation and cold exposure all produced substantial increases in adrenal TH activity (all Ps < .01). Each of these effects was independent of the cofactor concentration used (fig. 1).

Short-term effects. Abrupt hypotension produced either by treatment with isoproterenol (0.33 mg/kg s.c.) or phenoxybenzamine (20 mg/kg i.p.) resulted in a rapid and substantial increase in adrenal TH activity when assayed using a subsaturating concentration of cofactor (P < .01), whereas nonsignificant changes were observed when a saturating concentration was used instead (fig. 2). Similarly, glucoprivation resulting from administration of 2-deoxy-D-glucose (500 mg/kg i.p.) produced a rapid increase in TH activity when assayed using

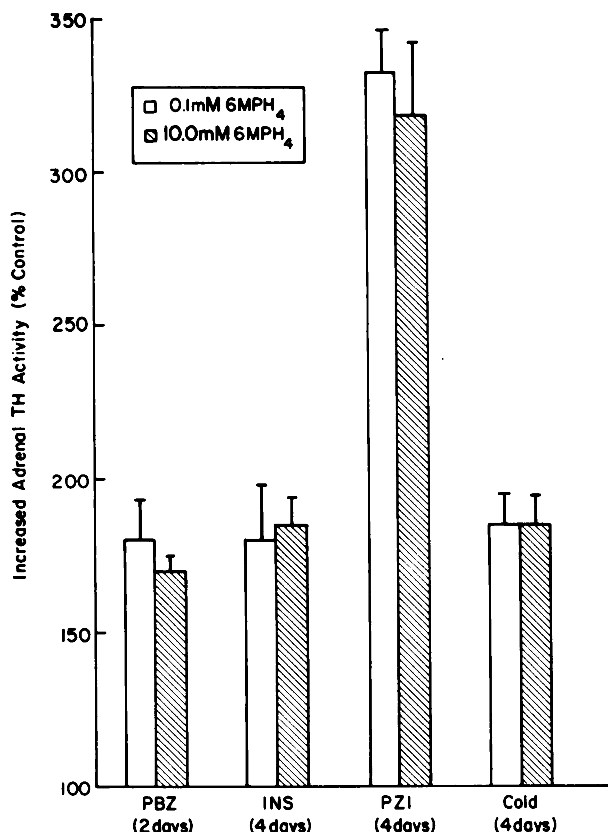

Fig. 1. Effect of chronic phenoxybenzamine (PBZ), insulin (INS), protamine-zinc insulin (PZI) or cold exposure on adrenal TH activity assayed in the presence of subsaturating (0.1 mM) and saturating (10.0 mM) concentrations of the cofactor (6-MPH$_4$). Values represent means ± S.E.M. based on five to eight animals and are expressed as a percentage of control values obtained on the day of the assay. These values ranged between 77 to 116 pmol/min/adrenal in the presence of subsaturating [6-MPH$_4$] and 526 to 700 pmol/min/adrenal in the presence of saturating [6-MPH$_4$].

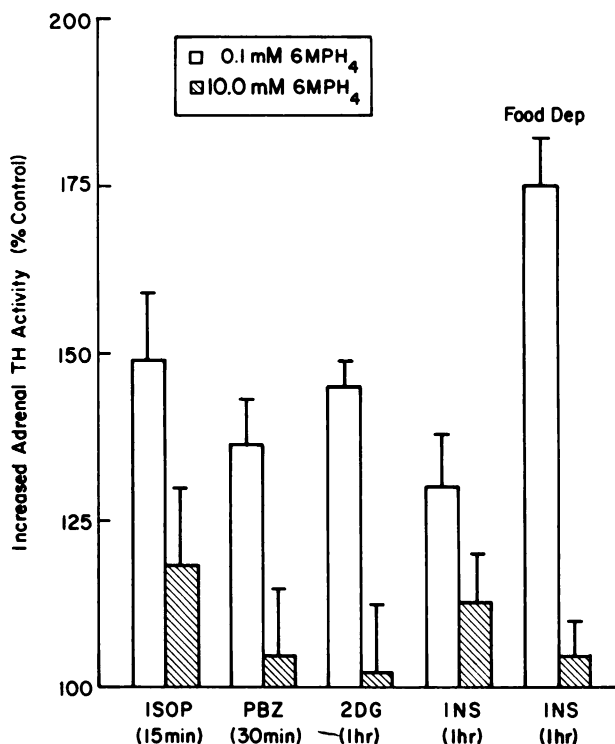

Fig. 2. Effect of acute isoproterenol (ISOP), phenoxybenzamine (PBZ), 2-deoxyglucose (2DG) and insulin (INS) on adrenal TH activity in the presence of subsaturating (0.1 mM) and saturating (10.0 mM) concentrations of the cofactor (6-MPH$_4$). Values represent means ± S.E.M. based on 6 to 10 animals and are expressed as a percentage of control values obtained on the day of the assay. These values ranged between 72 to 107 pmol/min/adrenal in the presence of subsaturating [6-MPH$_4$] and 466 to 700 pmol/min/adrenal in the presence of saturating [6-MPH$_4$].

subsaturating concentrations of cofactor (P < .01), but no change occurred when saturating concentrations were used (fig. 2).

Various insulin treatments were used to conduct a more complete analysis of the relation between the short-term activation of TH and the physiological impact of a stressor. Administration of regular insulin (20 U/kg s.c.) produced a fall in blood glucose levels to 47 mg/dl by 1 hr (P < .01) and an acute rise in TH activity (P < .05) that occurred only when a subsaturating concentration of cofactor was used in the assay (fig. 2). Prior food deprivation enhanced the induced hypoglycemia to 25 mg/dl after 1 hr, and adrenal TH activity increased correspondingly (P < .01; fig. 2). Finally, administration of varied doses of insulin produced graded degrees of hypoglycemia and proportionate elevations in TH activity ($r = -0.80$, P < .001; fig. 3), which were evident only when a subsaturating concentration of cofactor was used in the assay. Analysis of enzyme kinetics for 6-MPH$_4$ in the latter group of animals indicated that there was a significant decrease (P < .05) in the apparent K_m of adrenal TH for cofactor without a corresponding change in the apparent V_{max} (fig. 4).

In contrast to these effects of hypotension and glucoprivation, exposure to cold did not result in an acute activation of TH. Enzyme activity was not increased until after 48 hr of cold exposure (P < .01), at which time the apparent V_{max} of the enzyme was elevated substantially (fig. 5, lower panel). When the intensity of cold was enhanced by shaving the animals, the increase in TH activity during the first 4 days of exposure nevertheless was comparable to that seen in unshaved animals,

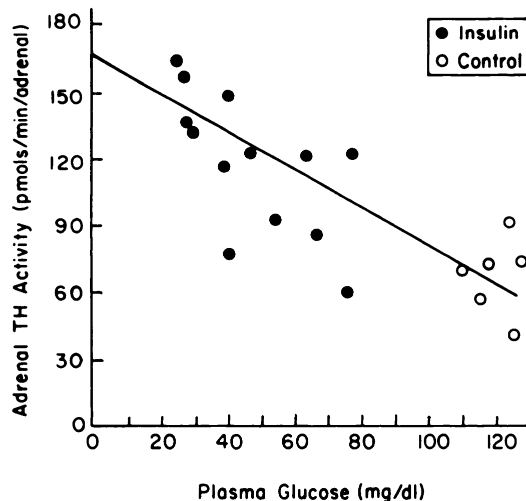

Fig. 3. Linear relation between plasma glucose concentration and adrenal TH activity as measured in the presence of subsaturating (0.1 mM) concentrations of the cofactor (6-MPH$_4$). Rats were sacrificed 1 hr after administration of insulin; some animals were food-deprived for 16 hr before insulin treatment in order to increase the severity of the hypoglycemia. (Each point represents a value obtained from one animal. Regression equation is: $y = -0.77x + 157.44$, $r = 0.80$, $t = 22.7$, $P < .001$).

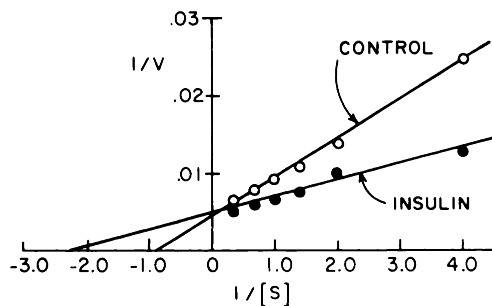

Fig. 4. Kinetic analysis of the effects of acute insulin administration on adrenal TH activity at various cofactor concentrations. The results are means from five separate experiments ± S.E.M. Apparent K_m for control tissue was 0.89 ± 0.10 mM whereas that of insulin-treated rats was 0.42 ± 0.01 mM. Apparent V_{max} was 690 ± 60 pmol/min/gland for both groups.

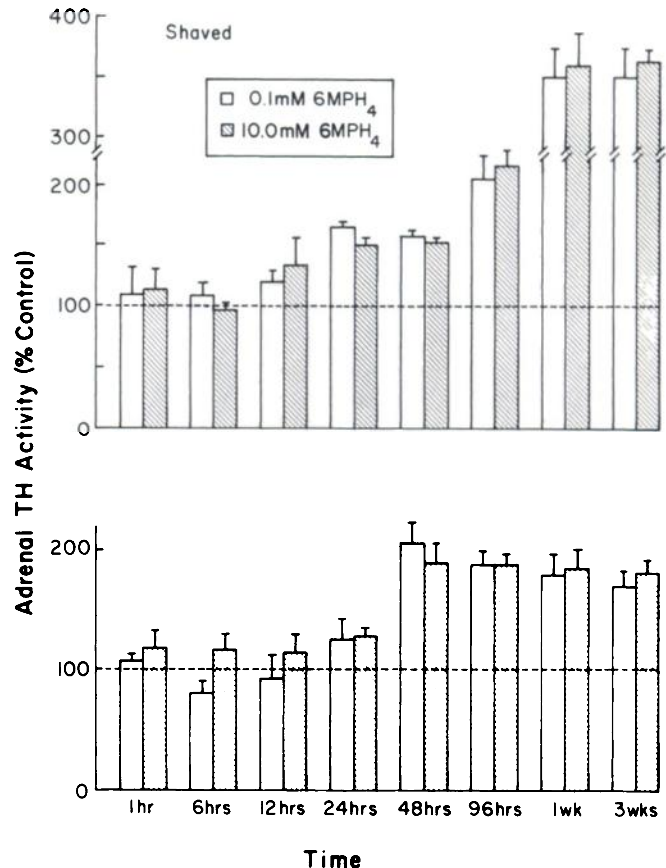

Fig. 5. Effect of various periods of cold exposure on adrenal TH activity assayed in the presence of subsaturating (0.1 mM) and saturating (10.0 mM) concentrations of the cofactor (6-MPH$_4$). Some animals were shaved before cold exposure (upper panel). Values represent means ± S.E.M. based on four to five animals and are expressed as a percentage of control values obtained on the day of the assays. Those values ranged between 84 to 116 pmol/min/adrenal in the presence of subsaturating [6-MPH$_4$] and 504 to 721 pmol/min/gland in the presence of saturating [6-MPH$_4$].

although after 1 and 3 weeks it reached values 2 to 3 times as large (fig. 5, upper panel). Thus, in neither the shaved nor the unshaved group of rats was there evidence that the enzyme had been activated within hours after the treatment began or at any time before the observed increase in maximal TH activity.

Adrenal denervation. It already is known that the long-term effect of cold stress on adrenal TH activity is abolished by adrenal denervation (Chuang *et al.*, 1975). To determine whether the short-term effects of insulin similarly were mediated transynaptically, we repeated those experiments in adrenal-denervated rats and found that the short-term activation of adrenal TH normally seen after insulin-induced hypoglycemia was prevented. As shown in figure 6, sham-operated control rats exhibited a 53% increase in adrenal TH activity ($P < .05$) 1 hr after administration of insulin (20 U/kg s.c.), when mean blood glucose was 51 mg/dl, whereas a comparable hypoglycemia (40 mg/dl) did not result in increased adrenal TH activity after adrenal denervation. These results are similar to the effects of adrenal denervation on the long-term induction of the enzyme after chronic cold exposure; 4 days of cold exposure induced a 40% increase in adrenal TH activity ($P < .05$) in sham-operated control rats but failed to do so in rats with denervated adrenal glands (fig. 6).

***In vivo* CA biosynthesis.** Because the short-term effect of cold exposure on adrenal TH activity was so different from that of insulin, we sought to determine whether the effects of these treatments on *in vivo* CA biosynthesis also differed. Acute administration of insulin (20 U/kg s.c.) was found to increase dopa accumulation ($P < .05$) in animals treated previously with RO 4-4602. Moreover, after 2 days of cold exposure, dopa levels also were elevated substantially ($P < .01$) (table 3). In both cases the percentage of rise in dopa accumulation above control levels was comparable to the changes observed in TH activity. However, short-term cold exposure did not increase dopa levels, suggesting that cold does not increase input to the adrenal gland until the exposure is prolonged.

6-Hydroxydopamine pretreatment. The absence of changes in *in vitro* TH activity and *in vivo* CA biosynthesis after acute cold exposure indicates that this stress does not stimulate the adrenal medulla in rats. However, because cold is well known to stimulate the sympathetic nerves (Leduc, 1961), it seemed possible that an adrenal medullary response to cold might be obtained if extensive damage to the sympathetic nerves had occurred previously. Accordingly, rats were given 6-hydroxydopamine (100 mg/kg s.c.) and 4 days later

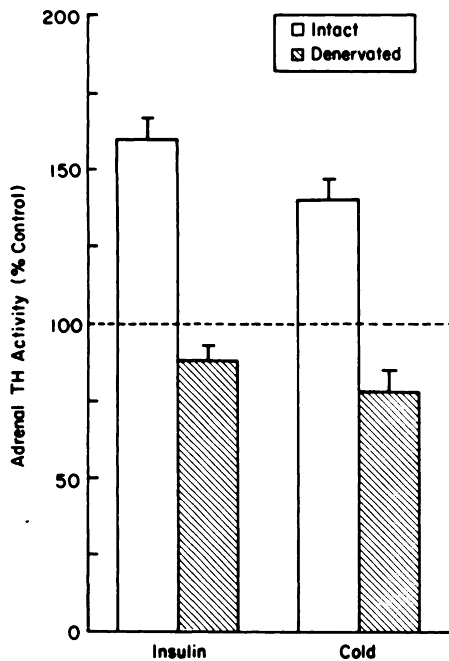

Fig. 6. Effect of adrenal denervation on adrenal TH activity after acute insulin administration (left panel) or chronic cold exposure (right panel). Rats were sacrificed 1 hr after insulin treatment and after 4 days of cold exposure. Adrenal TH activity was assayed in the presence of 1.0 mM 6-MPH$_4$. Values represent means ± S.E.M. based on 9 to 12 animals and are expressed as a percentage of control values obtained on the day of the assay, which ranged from 394 to 515 pmol/min/adrenal.

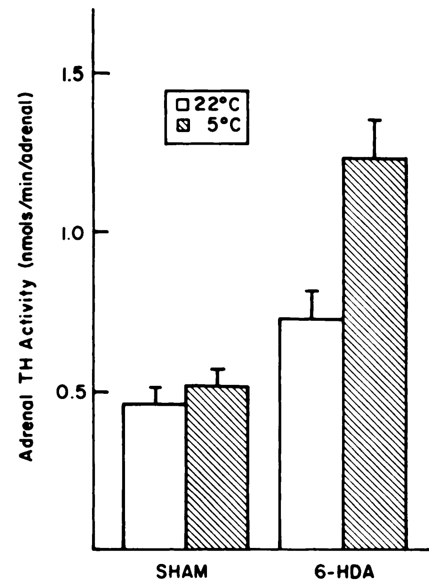

Fig. 7. Effect of pretreatment with 6-hydroxydopamine (6-HDA) on adrenal TH activity after acute cold exposure. Rats were treated with 6-HDA and 4 days later were exposed to 5°C for 1 hr before being sacrificed. Adrenal TH activity was assayed in the presence of 1.0 mM 6-MPH$_4$. Values represent means ± S.E.M. and are based on five to seven animals per group.

TABLE 3

Effect of insulin or cold exposure on adrenal levels of dihydroxyphenylalanine, epinephrine and norepinephrine

Values represent mean ± S.E.M. for six animals, 1 hr after treatment with the decarboxylase inhibitor RO4-4602. Dopa levels are expressed as nanograms per gland whereas epinephrine (EPI) and norepinephrine (NE) levels are micrograms per gland. Basal levels of dopa in eight nondrug treated animals were not detectable.

	Dopa	EPI	NE
Control	112.9 ± 6.1	9.6 ± 1.2	3.4 ± 0.7
Insulin (30 min)	147.9 ± 2.8*	8.7 ± 0.6	3.1 ± 0.2
Cold			
2 hr	103.4 ± 9.4	10.8 ± 0.7	3.5 ± 0.4
48 hr	181.8 ± 9.1**	8.2 ± 0.7	2.6 ± 0.1

* P < .05; ** P < .01, in comparison with control values.

TABLE 4

Effect of 6-hydroxydopamine (6-HDA) treatment on adrenal and cardiac CA levels

Values represent mean ± S.E.M. for five to seven animals. Adrenal levels of norepinephrine (NE) and epinephrine (EPI) are expressed as micrograms per gland, whereas cardiac levels are micrograms per gram. ND, not detectable.

	NE	EPI
Adrenal		
Control	3.5 ± 0.6	10.2 ± 1.3
6-HDA	4.3 ± 0.5	10.6 ± 1.5
Cardiac		
Control	0.637 ± 0.029	ND
6-HDA	0.049 ± 0.007**	ND

** P < .01, in comparison with control values.

norepinephrine content of the heart was found to be reduced by 93% (P < .01) whereas that of the adrenal was unaffected (table 4). In addition, adrenal TH activity was increased by 60% (P < .01), as has been reported previously (Mueller *et al.*, 1969; Thoenen *et al.*, 1969b). More significantly, when 6-hydroxydopamine-treated rats were exposed to cold for 1 hr, adrenal TH activity was elevated by an additional 55% (P < .01; fig. 7).

Discussion

Homeostatic challenges such as hypotension, hypoglycemia and cold exposure are well known stimuli of sympathoadrenal activity. The present experiments demonstrate that in response to the increased demands for CA release that they provoke, CA biosynthesis in the adrenal medulla apparently is increased by two temporally distinct processes: a rapid increase in the affinity of TH for cofactor and a more gradual increase in maximal TH activity most probably due to enzyme induction.

A rapid activation of TH has been observed previously both in the brain and the peripheral nervous system, in response to a variety of nonphysiological treatments. For example, it has been reported to occur in hippocampus after electrical stimulation of the locus coeruleus (Roth *et al.*, 1975), in striatum after systemic administration of antipsychotic drugs (Zivkovic *et al.*, 1974), and within the adrenal medulla after decapitation (Masserano and Weiner, 1979), electroconvulsive shock (Masserano *et al.*, 1981) and s.c. formalin treatment (Masserano and Weiner, 1981). In most cases this enzyme activation is characterized by a significant increase in the affinity of TH for its pterin cofactor. The present report demonstrates a similar increase in the affinity of adrenal TH for 6-MPH$_4$ after the acute onset of hypotension of hypoglycemia, disturbances that are severe but nonetheless physiological in nature.

Long-term changes in TH activity also have been described previously. For example, interruption of CA transmission by treatment with reserpine or 6-hydroxydopamine causes an induction of TH both in the brain and the periphery (Acheson and Zigmond, 1981; Reis *et al.*, 1975; Thoenen *et al.*, 1969b). In the present studies, chronic phenoxybenzamine, insulin or cold exposure similarly resulted in a gradual increase in the apparent V_{max} of adrenal TH. These results support previous proposals that an increase in TH protein occurs after repeated injections

of insulin (Patrick and Kirshner, 1971; Weiner and Mosimann, 1970) or phenoxybenzamine (Thoenen *et al.*, 1969a,b) and are consistent with immunoprecipitation studies indicating an increase in adrenal TH after cold stress (Chuang and Costa, 1974; Chuang *et al.*, 1975; Hoeldtke *et al.*, 1974). Moreover, they suggest that as an increase in maximal TH activity develops the increased affinity of the enzyme for cofactor disappears.

Previous reports (Chuang *et al.*, 1975; Thoenen *et al.*, 1969 b) have indicated that induction of adrenal TH is mediated transynaptically. In these studies, adrenal denervation abolished the increase in TH activity normally seen in the adrenal medulla after prolonged cold stress or treatment with either phenoxybenzamine or reserpine. The present experiments similarly demonstrated the necessity of an intact innervation for the apparent induction of adrenal TH after chronic cold exposure and add the new observation that the short-term activation of TH after acute insulin administration also is blocked by prior adrenal denervation.

Administration of either phenoxybenzamine or insulin resulted first in an activation of TH then was followed by a more gradually developing increase in the apparent V_{max} of the TH reaction. Cold exposure, on the other hand, produced a comparable change in V_{max} without evidence of an early activation or increase in *in vivo* CA biosynthesis. Moreover, prior shaving of the animals, which considerably enhanced the increase in V_{max}, still did not result in an early activation. It seems likely that these different stressors preferentially activate different components of the sympathetic nervous system; the counterregulatory responses to acute hypotension or hypoglycemia are known to be mediated, in part, by epinephrine secreted by the adrenal medulla, whereas the response to acute cold stress is mediated primarily by norepinephrine secreted from the sympathetic nerves (Lewis, 1975). Consistent with this proposal is evidence that the rise in plasma norepinephrine levels during acute cold exposure is not blocked by prior adrenal demedullation (Leduc, 1961; Picotti *et al.*, 1981) and that TH activity in sympathetic nerves is increased then (Fluharty *et al.*, 1984). Moreover, the present experiments indicate that acute exposure of rats to cold does activate adrenal TH when the sympathetic nerves had been destroyed by prior 6-hydroxydopamine treatment. Thus, the adrenal medulla may provide a reserve system for CA secretion during cold stress, to be used either when there are large and continuous needs for norepinephrine or when acute needs cannot be met by local sympathetic nerves.

The present studies of rats during cold stress demonstrate that induction of adrenal TH is not always preceded by an activation of the enzyme. These and other findings (Fluharty *et al.*, 1983; Masserano and Weiner, 1979) suggest that activation and induction may be independent processes. This hypothesis is supported by several recent reports describing the mechanisms by which the two processes are mediated. More specifically, Haycock *et al.* (1982) have suggested that activation of adrenal TH may be mediated by a Ca^{++}-dependent protein kinase, whereas the molecular mechanism mediating the induction of TH may involve a cyclic AMP-dependent protein kinase reaction with subsequent nuclear translocation (Costa and Guidotti, 1978). Although these hypotheses await further experimentation, they may provide the basis for the independence of activation and apparent induction of TH after stress.

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